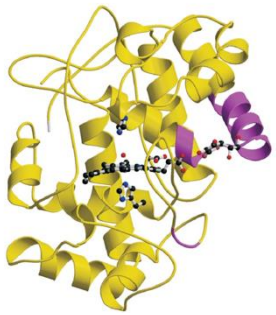
Design of new fluorescent sensors for measuring heme in cells

About the project or challenge area: Heme is essential for the survival of virtually all living systems - from bacteria, fungi and yeast, through plants to animals. No eukaryote has been identified that can survive without heme. It is involved in the control of many fundamental biological processes - such as oxygen transfer, redox control, respiration, photosynthesis, and drug metabolism - and has recently been found to have important regulatory/signalling roles, for example in transcriptional or circadian control. This ubiquitous presence in cells means that heme must be mobilised in response to cellular demands; it cannot simply diffuse around cells because heme is insoluble and it is also cytotoxic, so at high concentrations becomes a major problem to cells.



Structure of ascorbate peroxidase, to be used in this project.

The mechanisms by which cells control the supply and demand for heme and mobilise it to specific places are currently unknown. This lack of a generalised model for heme supply and demand is preventing, among other things, an understanding of how gene transcription is controlled in healthy cells or how certain pathways might be inappropriately activated in cells (including in disease).

The aim of this project is to precisely quantify heme concentrations at sub-cellular resolution, and under different conditions. We have developed a heme sensor technology that can be used to image cellular heme distributions in real time (using fluorescence lifetime imaging, FLIM), see publications. The sensor comprises the *apo*-form (*i.e.* without heme) of a monomeric form of ascorbate peroxidase (APX), referred to here as *apo*-mAPX, fused to a monomeric form of green fluorescent protein, mEGFP. The objectives are to design, express, purification and characterise these bespoke fluorescently-tagged heme sensors, and target them to different cellular regions. The outcomes of this programme will reveal where heme is located in cells, and how it responds to different stimuli, and thus will transform the understanding of the role of heme in biology. It will allow us to control and to visualise spatio-temporal changes in heme concentrations and distributions.

Why choose this opportunity? These methods for non-invasive measurement of intracellular heme will feed into the future development of therapeutic strategies that control or mitigate the disease consequences of excess or deficiencies in cellular heme. The project provides opportunities for a highly motivated student to be trained and gain skills in a wide range of disciplines, ranging from heme chemical biology, biophysics and cellular imaging, de novo protein design and computational modelling. The work is a collaboration with Prof Andrew Hudson at the University of Leicester, providing opportunities to network with experts in biophysical imaging. You will work closely with other members of the Bristol group, and you can expect to receive close mentoring in the preparation of scientific material for publication in peer-reviewed journals. You will participate with others in the group in external conferences (on-line), giving you an opportunity interact with other scientists outside of Bristol and to present your work to a wider audience.

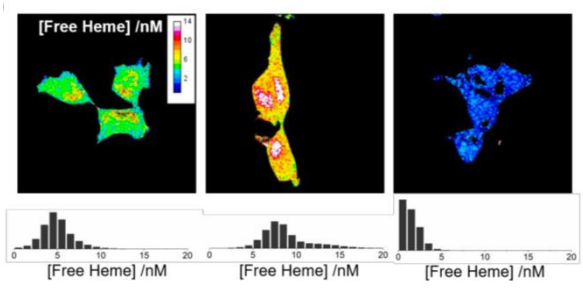


Figure 1. Imaging of heme in cells, using FLIM.

Full training will be provided for all aspects of this project. You will be embedded in Prof Raven's research group, and she will work closely with you to provide mentoring support. In addition, you will be assigned an additional mentor for the duration of your project, who will provide extra support and help you to identify any additional training needs or opportunities.

About you: You will have skills and knowledge in chemistry, chemical biology or biochemistry. You will be prepared to work well in a team, and be able to manage your time efficiently. These skills are desirable but not essential.

Bench fees: A bench fee of £7000 is required.

How to apply: Applications are accepted throughout the year and you should complete the online application form for Chemistry (MSc by Research).

Supervisor: Your supervisor for this project will be Professor Emma Raven in the School of Chemistry. You can contact her at +44 (0) 117 928 7657 or email emma.raven@bristol.ac.uk

Find out more about your prospective research program: Published work from the Raven lab below gives more information. *Science,* **2014***, 345*, 193-197 (DOI: 10.1126/science.1254398); *Proc. Natl. Acad. Sci.* **2016**, *113,* 3785-3790 (DOI: 10.1073/pnas.1600211113); *Proc. Natl. Acad. Sci USA* **2021**, 118 (22) 118 No. 22 e2104008118 (DOI: 10.1073/pnas.2104008118).